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Utility of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry following Introduction for Routine Laboratory Bacterial Identification[▽]

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Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) was evaluated prospectively in a diagnostic laboratory. Nine hundred twenty-seven organisms were tested in triplicate; 2,351/2,781 (85%) species and 2,681/2,781 (96%) genus identifications were correct. Known issues such as the misidentification of alpha-hemolytic streptococci as *Streptococcus pneumoniae* were easily corrected. Identifications cost AUD$0.45 per isolate and were available in minutes. MALDI-TOF MS is rapid, accurate, and inexpensive.

Matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS) has been shown to be both accurate in the identification of bacteria (1, 8, 9) and rapid (5, 10, 11, 13, 14), which is of proven benefit to patient care (2, 4). New technology is never able to completely replace conventional methods; rather, these tests form part of the overall diagnostic algorithms. We therefore undertook this prospective study to determine the utility of the MALDI-TOF MS in a routine diagnostic laboratory for bacterial identification.

All bacteria isolated within one calendar month from any site or specimen type that would normally undergo identification were tested in parallel with our routine methods and in triplicate (to assess the reproducibility of the results) using the microflex MALDI Biotyper 2.0 (Bruker Daltonics, GmbH, Bremen, Germany) according to the manufacturer's instructions (software version 3.1.1.0). As specified by the manufacturer, identification scores of ≥2 and between 1.7 and 1.9 were required for a reliable identification to the species and genus level, respectively, while identification scores of <1.7 were considered unreliable. MALDI-TOF MS identifications were compared to identifications by current methods, which included Vitek2 and API identification kits (bioMérieux, Australia) supplemented with conventional biochemical assays as required (e.g., oxidase, catalase). Resolution of discrepancies between results of conventional testing and the MALDI-TOF MS were initially made by repeating the MS test following crude extraction with formic acid using the manufacturer's recommended method. 16S rRNA sequencing was then used to resolve any remaining discrepancies. A result was considered a major error whenever the resolved final genus identification differed from that proposed by MALDI-TOF MS, while a result was considered a minor error when the genus identification was concordant but the species name was incorrect.

Nine hundred twenty-seven organisms were included in the study (Table 1) and tested in triplicate on the MALDI Biotyper (n = 2,781). Of these, 84.5% (2,351/2,781) and 96.4% (2,681/2,781) were correct to the species and genus level, respectively. In the 330 tests with identification scores between 1.7 and 1.9, the identification was correct to the species level in all cases. Furthermore, additional MS testing following crude extraction failed to produce an identification different from that originally proposed, although the identification score often increased to ≥2. Major errors occurred with one isolate of *Micrococcus luteus* (identified as *Staphylococcus simulans*) and all eight *Shigella* isolates (identified as *Escherichia coli*).

Minor errors occurred for one isolate of *Staphylococcus epidermidis* (identified as *Staphylococcus hominis*) and one isolate of *Enterobacter cloacae* (identified as *Enterobacter asburiae*), and the identifications remained incorrect despite repeated testing. All *Salmonella* isolates were identified by MALDI-TOF MS as *Salmonella* group isolates regardless of serotype. In addition, incorrect identification of the species occurred in one isolate of *Streptococcus agalactiae* (identified as *Streptococcus pneumoniae*) and six isolates of the *Streptococcus mitis* group (also identified as *Streptococcus pneumoniae*). In these cases, differentiation between incorrect and correct identifications was further aided by colony morphology and optochin testing.

Forty-eight of 2,781 (1.7%) tests gave unreliable results (score of <1.7). Provided the isolates were "common" bacteria (*Enterobacteriaceae*, Gram-positive cocci, and *Pseudomonas aeruginosa*), these could be resolved to the species level with repeated MALDI-TOF MS testing in 52% of cases (23/48). In contrast, bacteria infrequently encountered in our laboratory (e.g., Gram-positive rods or anaerobes) were unlikely to be resolved to the genus or species level with further testing by MALDI-TOF MS following crude extraction with formic acid.

Table 2 presents the comparison of total costs (reagents and

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TABLE 1. Performance of MALDI Biotyper in comparison to conventional testing for identification of commonly encountered bacteria in a microbiology laboratory

Bacterium	No. of isolates tested	No. of tests performed[a]	No. of MALDI Biotyper tests with[b]: Incorrect identification[c]	Unreliable identification	Correct identification with score of: 1.7–1.9	≥2	Proportion of tests correct to indicated level: Genus	Species
Anaerobes	12	36		1	12	23	0.97	0.64
Bacteroides fragilis	1	3				3	1.00	1.00
Bacteroides nordii	1	3			3		1.00	0.00
Bacteroides vulgatus	2	6				6	1.00	1.00
Bacteroides uniformis	1	3			2	1	1.00	0.33
Clostridium difficile	2	6			1	5	1.00	0.83
Clostridium perfringens	2	6				6	1.00	1.00
Finegoldia magna	1	3		1	1	1	0.67	0.33
Peptostreptococcus anaerobius	1	3			3		1.00	0.00
Prevotella bivia	1	3			2	1	1.00	0.33
Enterobacteriaceae	298	894	28	7	81	778	0.96	0.87
Citrobacter freundii	2	6				6	1.00	1.00
Citrobacter koseri	8	24			1	23	1.00	0.96
Enterobacter aerogenes	11	33		1		32	0.97	0.97
Enterobacter asburiae	3	9				9	1.00	1.00
Enterobacter cloacae	21	63	3		4	56	0.95	0.88
Escherichia coli	116	348		1	6	341	1.00	0.98
Klebsiella oxytoca	17	51		1		50	0.98	0.98
Klebsiella pneumoniae	44	132		2	4	126	0.98	0.95
Morganella morganii	4	12				12	1.00	1.00
Plesiomonas shigelloides	2	6			1	5	1.00	0.83
Proteus mirabilis	30	90		2	5	83	0.98	0.92
Providencia stuartii	2	6				6	1.00	1.00
Salmonella Typhimurium	20	60			60		1.00	0.00
Serratia marcescens	8	24	1		0	23	0.96	0.96
Shigella boydii	1	3	3				0.00	0.00
Shigella dysenteriae	1	3	3				0.00	0.00
Shigella flexneri	2	6	6				0.00	0.00
Shigella sonnei	4	12	12				0.00	0.00
Yersinia enterocolitica	2	6				6	1.00	1.00
Gram-positive rods	33	99		8	34	57	0.91	0.57
Arcanobacterium bernardiae	1	3				3	1.00	1.00
Arcanobacterium haemolyticum	1	3				3	1.00	1.00
Bacillus cereus	3	9			3	6	1.00	0.67
Clostridium innocuum	1	3		1	1	1	0.67	0.33
Corynebacterium amycolatum	2	6			1	5	1.00	0.83
Corynebacterium aurimucosum	1	3		1	2		0.67	0.00
Corynebacterium jeikeium	1	3		1		2	0.67	0.67
Dermabacter hominis	2	6			6		1.00	0.00
Dermatophilus congolensis	1	3			3		1.00	0.00
Erysipelothrix rhusiopathiae	1	3				3	1.00	1.00
Janibacter species	1	3		1	2		0.67	0.00
Lactobacillus sakei	1	3		1	1	1	0.67	0.33
Lactobacillus fermentum	1	3			2	1	1.00	0.33
Lactobacillus rhamnosus	3	9			9		1.00	0.00
Leuconostoc garlicum	1	3			3		1.00	0.00
Listeria monocytogenes	9	27			1	26	1.00	0.96
Raoultella planticola	1	3				3	1.00	1.00
Rhodococcus species	1	3		3			0.00	0.00
Rothia dentocariosa	1	3				3	1.00	1.00
Gram-positive cocci	429	1,287	24	31	160	1,072	0.95	0.83
Aerococcus urinae	1	3				3	1.00	1.00
Aerococcus viridans	1	3			1	2	1.00	0.67
Enterococcus casseliflavus	1	3				3	1.00	1.00
Enterococcus faecalis	37	111		2	11	98	0.98	0.88
Enterococcus faecium	25	75				75	1.00	1.00
Enterococcus gallinarum	2	6				6	1.00	1.00
Enterococcus raffinosus	1	3			2	1	1.00	0.33
Granulicatella adiacens	1	3		2	1		0.33	0.00
Micrococcus luteus	8	24	3		3	18	0.88	0.75
Staphylococcus aureus	146	438		5	22	411	0.99	0.94
Staphylococcus capitis	20	60		1	9	50	0.98	0.83
Staphylococcus cohnii	1	3			3		1.00	0.00
Staphylococcus epidermidis	36	108		3	25	80	0.95	0.72
Staphylococcus haemolyticus	2	6			1	5	1.00	0.83
Staphylococcus hominis	17	51			3	48	1.00	0.94
Staphylococcus lugdunensis	3	9		1	2	6	0.89	0.67
Streptococcus lutetiensis	1	3		1	2		0.67	0.00
Staphylococcus pettenkoferi	1	3				3	1.00	1.00
Staphylococcus saprophyticus	1	3				3	1.00	1.00

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TABLE 1—*Continued*

Bacterium	No. of isolates tested	No. of tests performed[a]	No. of MALDI Biotyper tests with[b]: Incorrect identification[c]	Unreliable identification	Correct identification with score of: 1.7–1.9	≥2	Proportion of tests correct to indicated level: Genus	Species
Staphylococcus warneri	5	15			10	5	1.00	0.33
Streptococcus agalactiae	22	66	3	2	5	56	0.93	0.85
Streptococcus anginosus	9	27		4	11	12	0.85	0.44
Streptococcus canis	1	3				3	1.00	1.00
Streptococcus constellatus	8	24		3	5	16	0.88	0.67
Streptococcus cristatus	2	6				6	1.00	1.00
Streptococcus dysgalactiae	15	45		2	20	23	0.96	0.51
Streptococcus gordonii	2	6		1	4	1	0.83	0.17
Streptococcus gallolyticus	1	3				3	1.00	1.00
Streptococcus mitis group	6	18	18		0	0	0.00	0.00
Streptococcus mutans	2	6				6	1.00	1.00
Streptococcus pneumoniae	25	75		2	12	61	0.97	0.81
Streptococcus pyogenes	19	57		1	2	54	0.98	0.95
Streptococcus salivarius	3	9		1	3	5	0.89	0.56
Streptococcus sanguinis	2	6				6	1.00	1.00
Streptococcus sinensis	1	3				3	1.00	1.00
Streptococcus vestibularis	1	3			3		1.00	0.00
Miscellaneous gram-negatives	78	234			18	216	1.00	0.92
Aeromonas hydrophila	2	6				6	1.00	1.00
Aeromonas veronii	2	6				6	1.00	1.00
Aggregatibacter aphrophilus	2	6			3	3	1.00	0.50
Campylobacter coli	1	3			1	2	1.00	0.67
Capnocytophaga ochracea	1	3			2	1	1.00	0.33
Eikenella corrodens	3	9			3	6	1.00	0.67
Haemophilus influenzae	32	96			2	94	1.00	0.98
Haemophilus parainfluenzae	1	3			1	2	1.00	0.67
Helcococcus kunzii	1	3				3	1.00	1.00
Kingella kingae	1	3				3	1.00	1.00
Moraxella catarrhalis	3	9			3	6	1.00	0.67
Neisseria elongata	1	3			3		1.00	0.00
Neisseria gonorrhoeae	14	42				42	1.00	1.00
Neisseria meningitidis	13	39				39	1.00	1.00
Pasteurella multocida	1	3				3	1.00	1.00
Non-fermentative gram-negative rods	77	231		1	25	205	1.00	0.89
Acinetobacter baumannii	2	6				6	1.00	1.00
Acinetobacter lwoffii	5	15			2	13	1.00	0.87
Achromobacter xylosoxidans	3	9			9		1.00	0.00
Agrobacterium tumefaciens	1	3			3		1.00	0.00
Burkholderia cepacia	7	21				21	1.00	1.00
Myroides odoratimimus	1	3				3	1.00	1.00
Ochrobactrum anthropi	1	3				3	1.00	1.00
Photorhabdus asymbiotica	1	3				3	1.00	1.00
Pseudomonas aeruginosa	44	132		1		131	0.99	0.99
Pseudomonas geniculata	2	6			4	2	1.00	0.33
Pseudomonas hibiscicola	2	6			1	5	1.00	0.83
Pseudomonas koreensis	1	3				3	1.00	1.00
Pseudomonas putida	1	3				3	1.00	1.00
Roseomonas gilardii	1	3				3	1.00	1.00
Roseomonas mucosa	1	3				3	1.00	1.00
Shewanella putrefaciens	1	3				3	1.00	1.00
Stenotrophomonas maltophilia	3	9			6	3	1.00	0.33
Total	927	2781	52	48	330	2351	0.96	0.84

[a] All isolates were tested in triplicate.

[b] Results were compared to those of conventional testing, with concordant results accepted as the correct identification. Isolates with discordant results were retested following crude extraction with formic acid, and if unresolved, 16S rRNA sequencing was used to confirm the identification. An unreliable identification was defined by a score of ≤1.7. In accordance with the manufacturer's specifications, identification scores of ≥2 and between 1.7 and 1.9 were required for a reliable identification to the species or genus level, respectively.

[c] Incorrect identifications include major (incorrect genus) and minor (correct genus, incorrect species) errors.

labor) to result for the 927 isolates examined during this evaluation. The cost of current methodologies ranges, in Australian dollars, from AUD$1.55 (staphylococcal latex and DNase) to AUD$31.76 (organisms requiring conventional tube biochemistry and/or serological confirmation). The average cost for the identification of a coliform by Vitek2 is AUD$10.00. In comparison, the MALDI-TOF costs are standard for all organism groups, with reagent and labor costs of AUD$0.45. A further advantage of the MALDI-TOF MS is that results are available up to 20 h earlier than results of conventional testing, which can take 18 h for staphylococci and 48 h for nonfermentative Gram-negative bacilli.

Overall, the results of our evaluation support those previously published, verifying the accuracy of MALDI-TOF MS as a means of performing rapid bacterial identifications (5, 10, 11, 14). However, our experience would indicate that the MALDI

TABLE 2. Comparison of total cost to result for 927 clinical bacterial isolates[a]

Organism group	No. of isolates	Cost (AUD$)		Cost savings per group (AUD$)
		Current method	Using MALDI-TOF MS[b]	
Anaerobes	12	126.00	5.40	120.60
Enterobacteriaceae	298	3,314.64	1,010.78[c]	2,303.86
Gram-positive rods	33	833.91	344.85[d]	489.06
Gram-positive cocci	429	3,714.21	527.56[e]	3,186.65
Miscellaneous gram-negatives	78	1,471.82	35.10	1,436.72
Non-fermentative gram-negative rods	77	893.43	34.65	858.78
Total	927	10,354.01	1,958.34	8,395.67

[a] Includes reagent and labor costs based upon one identification attempt per isolate.
[b] Only six isolates required crude extraction to provide an acceptable identification. This added an additional AUD$2.70 to the total cost of the MALDI-TOF identifications.
[c] Includes the cost of conventional identification methods (including serological confirmation) for 28 isolates that could not be identified by MALDI-TOF MS.
[d] Includes the cost for the identification of three isolates by 16S rRNA analysis.
[e] Includes the cost for additional identification methods such as optochin susceptibility for 38 isolates.

Biotyper, like any identification system, has limitations even for common clinical isolates, and the identifications obtained must be interpreted by experienced laboratory personnel in conjunction with clinical presentation and colonial morphology. For example, as detailed above, the misidentification of six isolates of *S. mitis* as *S. pneumoniae* was readily recognized as incorrect, due to the absence of typical pneumococcal colonial morphology and optochin resistance. Recognition of this limitation is particularly important when using this instrument to identify bacteria directly from positive blood culture broths when colonial morphology is not immediately available (3).

Our results also indicate that isolates of non-lactose-fermenting or slowly lactose-fermenting *Enterobacteriaceae* isolated from enteric specimens and identified as *E. coli* by the MALDI Biotyper will require both conventional biochemical and serological identification to exclude *Shigella* species. Similarly, while organisms are reliably identified as *Salmonella* species, additional biochemical and serological testing will be required to exclude *Salmonella enterica* serotypes Typhi and Paratyphi, the differentiation of which is of clinical and public health importance.

In contrast, for "common" bacteria, we found the manufacturer's recommendations overly stringent, such that identification scores between 1.7 and 1.9 could be used to reliably identify bacteria to the species level, particularly when supported by typical cultural morphology. In addition, it was apparent that "uncommon" isolates that gave unreliable results were not able to be resolved with further testing by the MALDI Biotyper (even following crude extraction), and this may reflect deficiencies in the current database.

Crude extraction is not routinely required, as the majority of clinical isolates were correctly identified without it. However, subsequent experience has indicated that crude extraction at the time of first testing increases the reliability of the identification to the species level of non-*Enterococcus faecalis* enterococci.

Consequently, clinical laboratories wishing to implement MALDI-TOF MS technology will need to establish appropriate testing algorithms in light of the recognized strengths and limitations. These algorithms should be devised in conjunction with conventional laboratory considerations such as specimen type and colony morphology where chromogenic and selective agars may prove to be an important adjunct (7). It must also be remembered that MALDI-TOF MS is not currently able to determine susceptibility patterns for clinical isolates, and other methods or instrumentation is still required to perform this task.

In conclusion, although the cost for the outright purchase of the instrument is considerable (approximately AUD$200,000), the MALDI Biotyper offers routine clinical laboratories a reliable, accurate, and cost-effective means for identifying a broad range of bacteria. Its simple procedures afford laboratories the possibilities of improved workflow and efficiency, along with considerable savings on consumables, reagents, and labor. In our laboratory, these savings will offset the initial purchase price within 3 years. While the benefits of rapid results to patient care are already apparent, the adaptation and validation of the technology to direct specimen analysis will only increase the clinical benefits into the future (2, 3, 4, 6, 12).

REFERENCES

1. **Anhalt, J. P., and C. Fenselau.** 1975. Identification of bacteria using mass spectrometry. Ann. Chem. **47:**219–225.
2. **Barenfanger, J., C. Drake, and G. Kacich.** 1999. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. J. Clin. Microbiol. **37:**1415–1418.
3. **Christner, M., et al.** 2010. Rapid identification of bacteria from positive blood culture bottles by use of matrix-assisted laser desorption ionization—time of flight mass spectrometry fingerprinting. J. Clin. Microbiol. **48:**1584–1591.
4. **Doern, G. V., R. Vautour, M. Gaudet, and B. Levy.** 1994. Clinical impact of rapid *in vitro* susceptibility testing and bacterial identification. J. Clin. Microbiol. **32:**1757–1762.
5. **Eigner, U., et al.** 2009. Performance of a matrix-assisted laser desorption ionization-time-of-flight mass spectrometry system for the identification of bacterial isolates in the clinical routine laboratory. Clin. Lab. **55:**289–296.
6. **Ferreira, L., et al.** 2010. Direct identification of urinary tract pathogens from urine samples by matrix-assisted laser desorption ionization—time-of-flight mass spectrometry. J. Clin. Microbiol. **48:**2110–2115.
7. **He, Y., H. Li, X. Lu, C. W. Stratton, and Y.-W. Tang.** 2010. Mass spectrometry biotyper system identifies enteric bacterial pathogens directly from colonies grown on selective stool culture media. J. Clin. Microbiol. **48:**3888–3892.
8. **Holland, R. D., et al.** Jr. 1996. Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. **10:**1227–1232.
9. **Krishnamurthy, T., P. L. Ross, and U. Rajamani.** 1996. Detection of pathogenic and non-pathogenic bacteria by matrix-assisted laser desorption/ion-

ization time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. **10:**883–888.
10. **Marklein, G., et al.** 2009. Matrix-assisted laser desorption ionization–time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J. Clin. Microbiol. **47:**2912–2917.
11. **Mellmann, A., et al.** 2008. Evaluation of matrix-assisted laser desorption ionization–time-of-flight mass spectrometry in comparison to 16S rRNA gene sequencing for species identification of nonfermenting bacteria. J. Clin. Microbiol. **46:**1946–1954.
12. **Prod'hom, G., A. Bizzini, C. Durussel, J. Bille, and G. Greub.** 2010. Matrix-assisted laser desorption ionization—time of flight mass spectrometry for direct bacterial identification from positive blood culture pellets. J. Clin. Microbiol. **48:**1481–1483.
13. **Seng, P., et al.** 2009. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin. Infect. Dis. **49:**543–551.
14. **van Veen, S. Q., E. C. J. Claas, and E. J. Kuijper.** 2010. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization–time of flight mass spectrometry in conventional medical microbiology laboratories. J. Clin. Microbiol. **48:**900–907.